Nuclease

Week 4

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 Gel electrophoresis of T7-Nuc

Responsible

Oscar He, Ellinor Lindholm

Experimental Setup

a. Prepare a gel for troubleshooting of T7-Nuc samples.

b. Gel electrophoresis of the above mentioned samples with additional 1:5 and 1:10 dilutions of the samples according to the table below:

Table 1: Dilutions of T7-Muc

Dilution	**DNA (μl)**	**dH_2O (μl)**	**Loading dye (μl)**
Original sample	4	0	1
1:5	1	4	1.25
1:10	0.5	4.5	1.25

Results and Conclusions

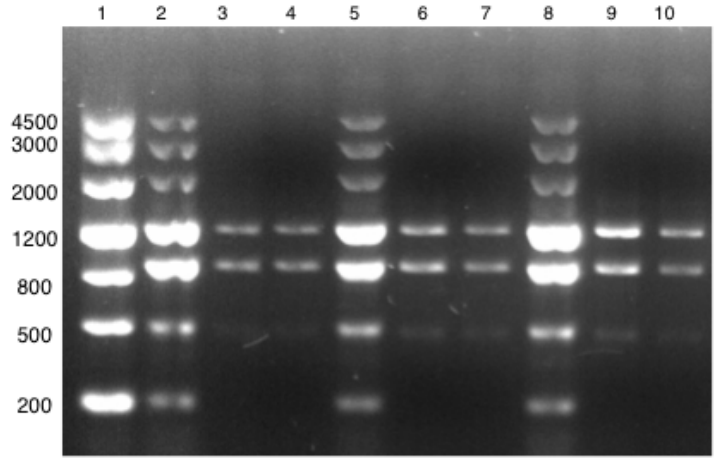

Figure 1: Gel of T7-Nuc PCR products.

Conclusion: Seems like our Nuc samples contain - or actually are - Esp (these tests were run in parallel).

Discussion and Troubleshooting

It seems like our Nuc samples contain - or actually are - Esp (these tests were run in parallel). We are unsure as to how this contamination occurred but will redo the 3A-Assembly.

2 Digestion of Nuc

Responsible

Bethel Tesfai Embaie

Protocols used

3A Assembly

Experimental set up

The digestion followed the digestion protocol. See table below for Sample structure.

Table 2: Labeling of samples

Nuc 2	**N1**	**N2**	**N3**	**N4**	**N5**	**N6**
Conc 75.8 ng/μl	sd XbaI	sd PstI	dd	dd	dd	undigested negative control

Table 3: Enzyme Master Mixes

Enzyme MM XbaI	**Volume [μl]**	**Enzyme MM PstI**	**Volume [μl]**
Total Volume	16	Total Volume	16
10x cutsmart	1.6	10x cutsmart	1.6
ddH_2O	13.6	ddH_2O	13.6
XbaI enzyme	0.8	PstI enzyme	0.8

Table 4: Nuc Digestions

Single digestion	**μl**	**Double digestion**	**μl**
DNA sample	1.32	DNA sample	1.32
Enzyme MasterMix	2	Enzyme MasterMix 1	2
10x cutsmart	0.6	Enzyme MasterMix 2	2
ddH_2O	4.08	10x cutsmart	0.4
		ddH_2O	2.28

Gel: 8ul digestion products were mixed with 2ul loading dye and run on the gel.

Sample Calculation

The volume of DNA sample to add to the digestion mix

$$Volume\ of\ DNA\ sample = \frac{Final\ amount\ of\ DNA}{Concentration\ of\ DNA\ in\ sample} \quad (1)$$

Results and Conclusions

Table 5: Labeling of gel

Well	Component	Comment
1	DNA ladder	
2	N1 sd XbaI	successful digestion
3	N2 sd PstI	successful digestion
4	N3 dd	successful digestion
5	N4 dd	successful digestion
6	N5 dd	successful digestion
7	N6 negative control	

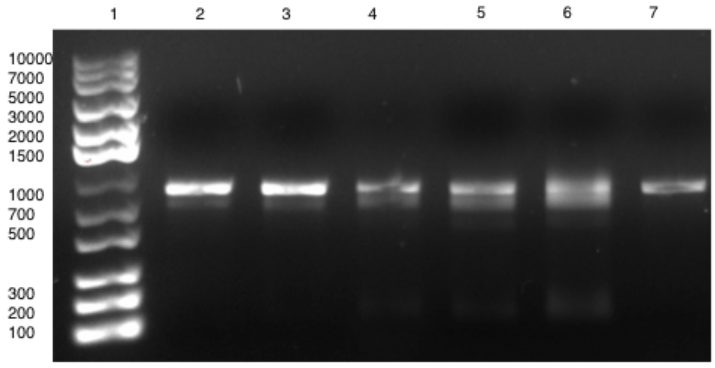

Figure 2: Gel of digested Nuc

Discussion and Troubleshooting

The digestion was successful and the experiment was continue with ligation and transformation.

3 Gel purification of Nuc

Responsible

Bethel Tesfai Embaie

Protocols used

Qiagen Gel Extraction kit

Modifications to protocols

We used prepared Isopropanol from fume-hood. We performed step 6, and let QG buffer stand for a minute. Then we added 25ul EB elution buffer and left to stand for 4 minutes.

Experimental set up

Extract more DNA from digested Nuc for 3A assembly ligation.

Sample Calculation

Table 6: Summary of experimental set up

Gel sample	**Nuc 3**	**Nuc 4**	**Nuc 5**
Weight of gel:	263.9 mg	229.4 mg	293.1 mg
Added QG buffer 3x vol:	791.7 μl	688.2 μl	879.3 μl
isopropanol 1 volume:	263.9 μl	229.4 μl	293.1 μl

Results and Conclusions

DNA was extracted and the nanodrop showed a Nuc concentration of 4.4 ng/μl.

4 Gel purification of double digested backbone

Responsible

Sigrun Stulz

Protocols used

Qiagen Gel Extraction kit

Experimental set up

Weighed each gel
Added QG buffer 3x vol
isopropanol 1 volume
Combined both samples for centrifugation
Re-elution performed due to low concentration on nanodrop

Results and Conclusions

Table 7: Results of nanodrop

Nanodrop	**Concentration**
Vector	2.7 ng/μl
Rediluted vector	3.5 ng/μl

Discussion and Troubleshooting

The DNA concentrations were very low and do not correspond to the typical yields of gel purifications, In a next attempt, more DNA could be loaded on the gel from the beginning.

5 Ligation of pSB1C3-T7-Nuc

Responsible

Bethel Tesfai Embaie

Protocols used

- 3A Assembly
- Digestion and ligation

Modifications to protocols

3A Assembly (ligation):	Amount of vector per reaction	50 ng
	Ratio vector:T7	1:3
	Ratio vector:Nuc2	1:3
	Incubation:	RT for 20 min, heat kill 80 °Cfor 20 min.

Experimental Setup

Table 8: Concentration and size of the parts used for the ligation in the 3A assembly.

Assemble part	Concentration [ng/μl]	Size [bp]
pSB1C3	11.5	2155
T7	6.52	55
Nuc2	4.4	561

Table 9: Volumes of each component used in the ligation mix.

Assemble part	Nuc2 [μl]
pSB1K3	data lost
T7	data lost
DNA Sample	data lost
T4 Ligase	1
10x T4 DNA Ligase Buffer	4
dH_2O	data lost

Sample Calculation

Moles of vector and mass of insert per reaction were calculated with NEBioCalculator^{TM}.

Volume of pSB1A3, T7 and nuclease

$$Volume\ of\ vector = \frac{Amount\ of\ vector\ per\ reaction}{Concentration\ of\ vector\ sample} \tag{2}$$

$$Volume\ of\ insert = Ratio\ \cdot\ \frac{Amount\ of\ insert\ per\ reaction}{Concentration\ of\ insert\ sample} \tag{3}$$

Results and Conclusions

Gel 1: ran for too long; we lost digested Nuc.
Gel 2: we repeated the gel with the remaining T7 digestion products.

Table 10: Gel labelling

	Label	**Comment**
1	DNA ladder	
2	T7 sd EcoRI	successful
3	T7 sd SpeI	successful
4	T7 dd	similar length; could be used for ligation
5	T7 dd	similar length; could be used for ligation
6	T7 dd	similar length; could be used for ligation
7	T7 negative control	looks right
8	ladder	

Discussion and Troubleshooting

The nuc gel ran for too long and the samples were unfortunately lost. Hence we need to amplify more Nuc and run a new gel.

6 Plasmid purification of T7-Nuc

Responsible

Ellinor Lindholm, Oscar He

Protocols used

QIAGEN MiniPrep

Experimental Set up

Purification was made of 3A assembled plasmids containing T7 promotor and Nuc/Lys.
After purification concentration measurement was performed using the Nanodrop

Sample Calculation

The final concentration of plasmid sample in PCR mix

$$Final\ concentration = \frac{Initial\ concentration}{Dilution\ factor} \tag{4}$$

Results and Conclusions

Table 11: Concentrations of purified plasmids

Sample	**Concentration (ng/μl)**
T7-Nuc 2.1	79
T7-Nuc 2.2	40
T7-Nuc 2.3	44

Discussion and Troubleshooting

Only one of the plasmids (sample T7-Nuc 2.1) had high enough concentration to be used for further analysis. In the following pages, this is referred to as T7-Nuc.

7 Gel electrophoresis of T7-Nuc

Responsible

Oscar He, Ellinor Lindholm

Protocols used

Gel Electrophoresis

Experimental Set up

The aim was to run a gel electrophoresis of the Nuc samples with additional 1:5 and 1:10 dilutions.

Table 12: My caption

Dilution	**DNA**	**dH_2O**	**Loading dye**	**Units**
Original sample	4	0	1	μl
1:5	1	4	1.25	μl
1:10	0.5	4.5	1.25	μl

Results and Conclusions

Table 13: Gel labeling

Well	**Component**	**Comment**
1	DNA Ladder	
2	Nuc2.1, 1:1	Ladder contamination
3	Nuc2.1, 1:5	
4	Nuc2.1, 1:10	
5	Nuc2.2, 1:1	Ladder contamination
6	Nuc2.2, 1:5	
7	Nuc2.2, 1:10	
8	Nuc2.3, 1:1	Ladder contamination
9	Nuc2.3, 1:5	
10	Nuc2.3, 1:10	
11	DNA Ladder	

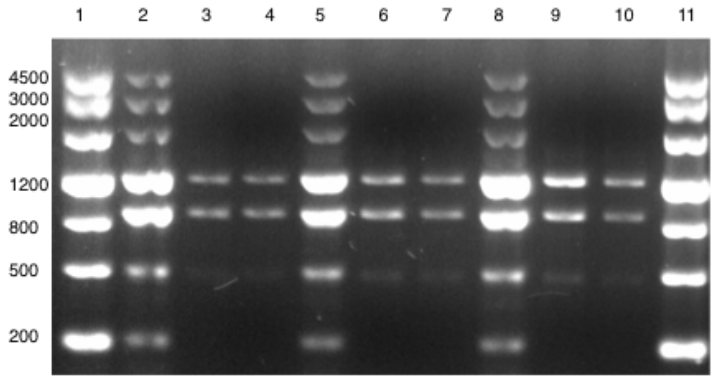

Figure 3: Gel of T7-Nuc PCR products.

Discussion and Troubleshooting

Seems like Nuc contains - or actually is - Esp.